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53. The plant cell of claim 52, wherein said fourth coding sequence comprises at least a portion of the nucleotide sequence set forth in SEQ ID NO: 24.

5 54. The plant cell of claim 52 further comprising in its genome a fifth DNA construct comprising a promoter that drives expression in a plant cell operably linked to a fifth coding sequence, wherein said fifth coding sequence encodes an NADH kinase or an NAD<sup>+</sup> kinase and said fifth coding sequence is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal.

10

55. The plant cell of claim 54, wherein said fifth coding sequence comprises at least a portion of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25-27.

15 56. A plant cell genetically manipulated to produce polyhydroxyalkanoate in its peroxisomes, said plant cell comprising in its genome:

a stably integrated first DNA construct comprising a promoter that drives expression in a plant cell operably linked to a first coding sequence, wherein said first coding sequence encodes a polyhydroxyalkanoate synthase and is operably  
20 linked to a nucleotide sequence encoding a peroxisome-targeting signal;

a stably integrated second DNA construct comprising a promoter that drives expression in a plant cell operably linked to a second coding sequence, wherein said second coding sequence encodes an acetyl-CoA:acetyl transferase and is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal; and

25 a stably integrated third DNA construct comprising a promoter that drives expression in a plant cell operably linked to a third coding sequence, wherein said third coding sequence is operably linked to a nucleotide sequence encoding a peroxisome-targeting signal and said third coding sequence is selected from the group consisting of:

- 30 (a) a nucleotide sequence encoding a 3-ketoacyl-CoA reductase that is capable of utilizing NADH;
- (b) a nucleotide sequence set forth in SEQ ID NO: 3;
- (c) a nucleotide sequence set forth in SEQ ID NO: 22;

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- (d) the nucleotide sequence set forth in SEQ ID NO: 1;
- (e) a nucleotide sequence encoding a multifunctional protein-2, wherein the hydratase activity of said multifunctional protein has been eliminated; and
- 5 (f) the nucleotide sequence set forth in SEQ ID NO: 6.

57. The plant cell of claim 56, wherein said second coding sequence comprises at least a portion of the nucleotide sequence set forth in SEQ ID NO: 24.

10 58. A plant cell genetically manipulated for the synthesis in its peroxisomes of at least one intermediate molecule in polyhydroxyalkanoate synthesis, said plant cell comprising in its genome at least one stably incorporated DNA construct comprising a coding sequence for an enzyme involved in the synthesis of said intermediate molecule, said coding sequence operably linked to a promoter that  
15 drives expression in a plant cell and to a nucleotide sequence encoding a peroxisome-targeting signal, wherein said coding sequence is selected from the group consisting of:

- (a) a nucleotide sequence encoding a 2-enoyl-CoA hydratase that is capable of catalyzing the synthesis of  
20 R-(-)-3-hydroxyacyl-CoA ;
- (b) a nucleotide sequence set forth in SEQ ID NO: 21;
- (c) a nucleotide sequence comprising the 2-enoyl-CoA hydratase domain of a multifunctional protein-2.
- (d) a nucleotide sequence set forth in SEQ ID NO: 4;
- 25 (e) a nucleotide sequence set forth in SEQ ID NO: 1;
- (f) a nucleotide sequence encoding a multifunctional protein-2, wherein the dehydrogenase activity of said multifunctional protein has been eliminated; and
- (g) a nucleotide sequence encoding a 3-ketoacyl-CoA reductase that is capable of utilizing NADH;
- 30 (h) a nucleotide sequence set forth in SEQ ID NO: 3;
- (i) a nucleotide sequence set forth in SEQ ID NO: 22;
- (j) the nucleotide sequence set forth in SEQ ID NO: 1;

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- (k) a nucleotide sequence encoding a multifunctional protein-2, wherein the hydratase activity of said multifunctional protein has been eliminated; and
- (l) the nucleotide sequence set forth in SEQ ID NO: 6.

5

59. The plant cell of claim 58, wherein said intermediate molecule is an R-(-)-3-hydroxyacyl-CoA or a 3-ketoacyl-CoA.

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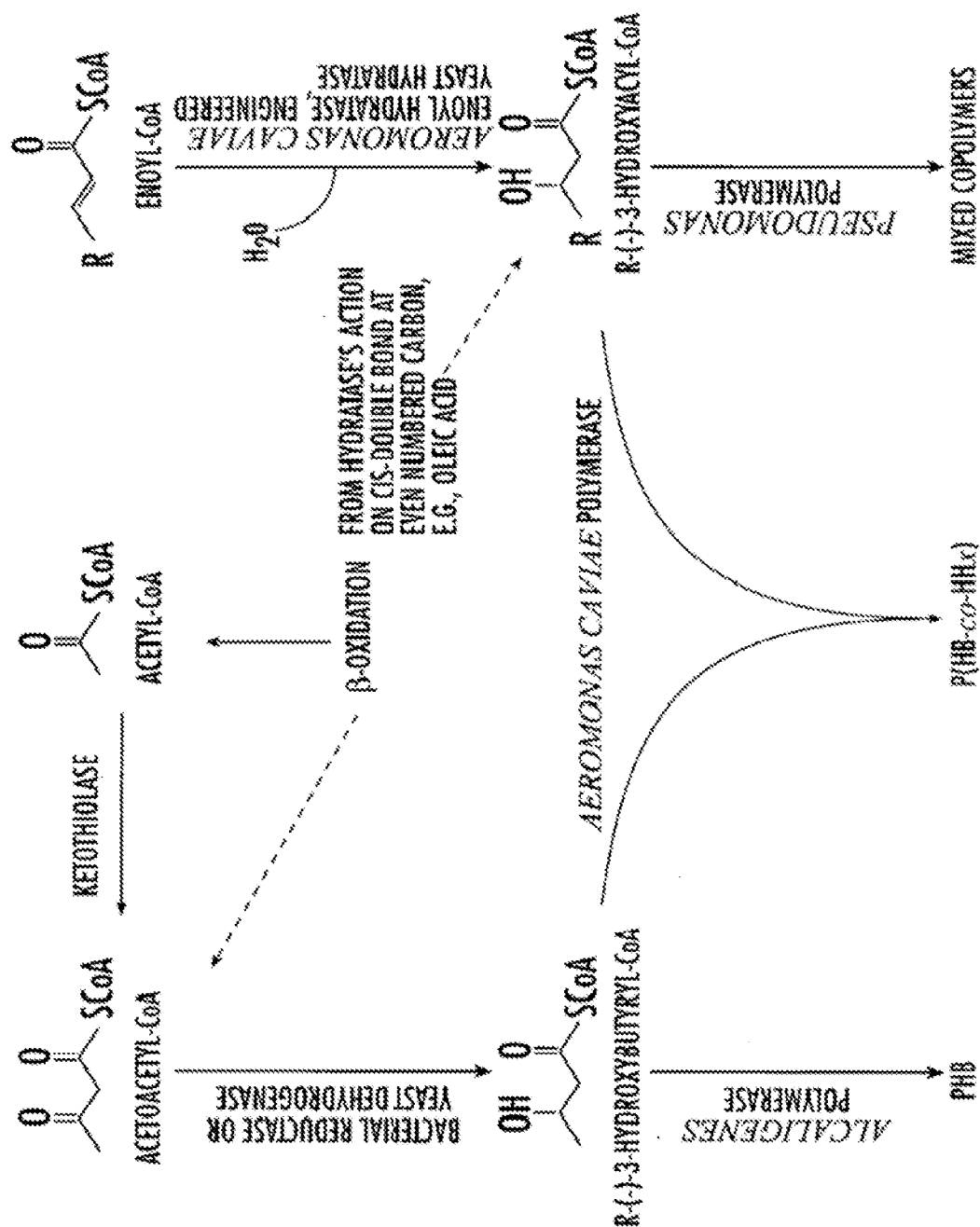


FIG. 1.

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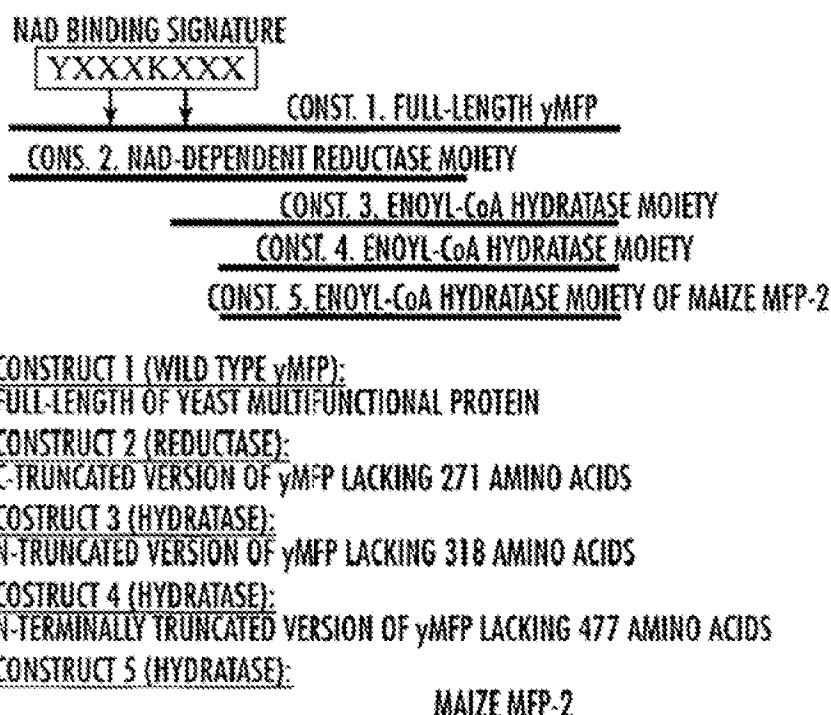


FIG. 2

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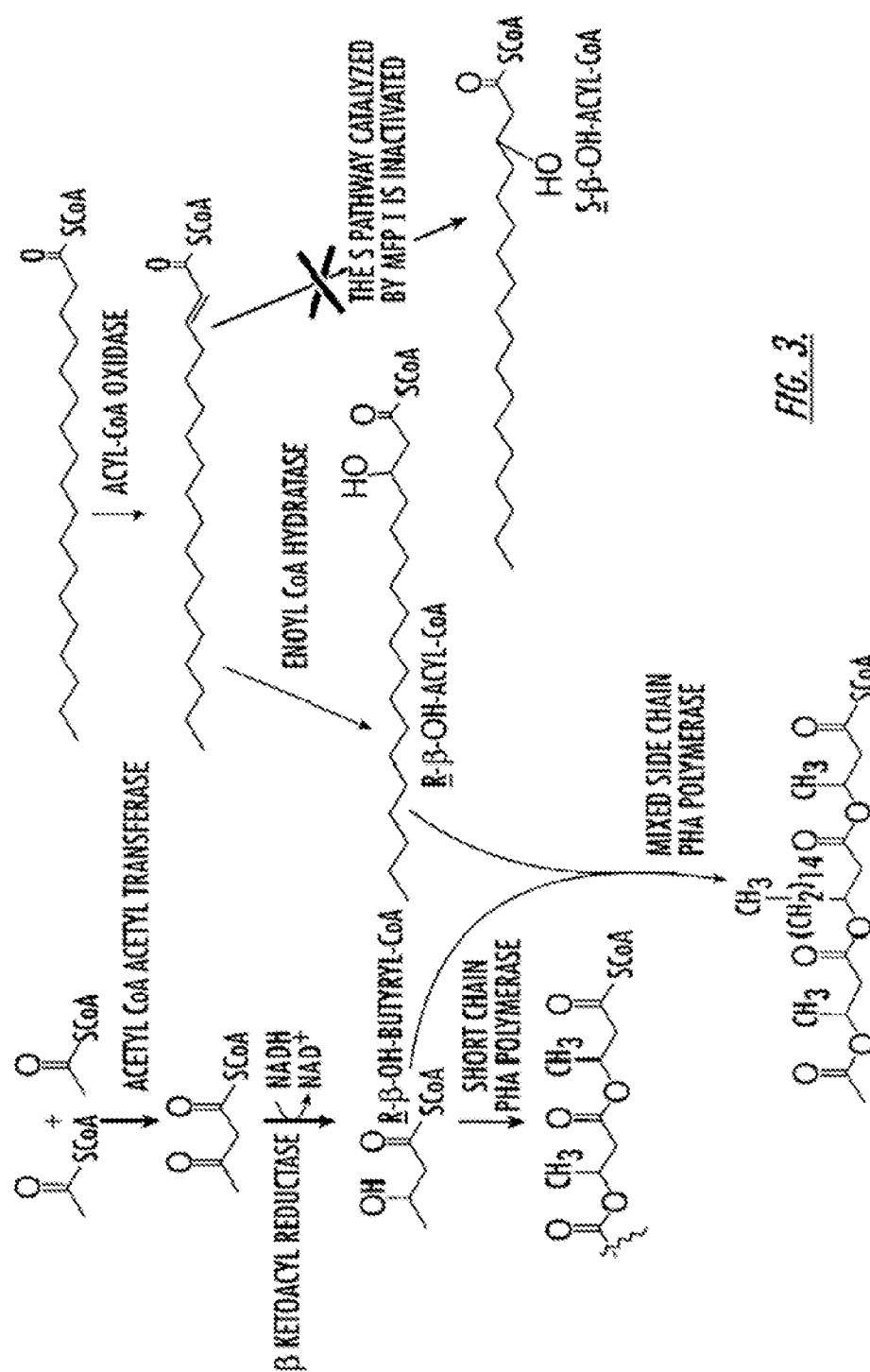


FIG. 3.

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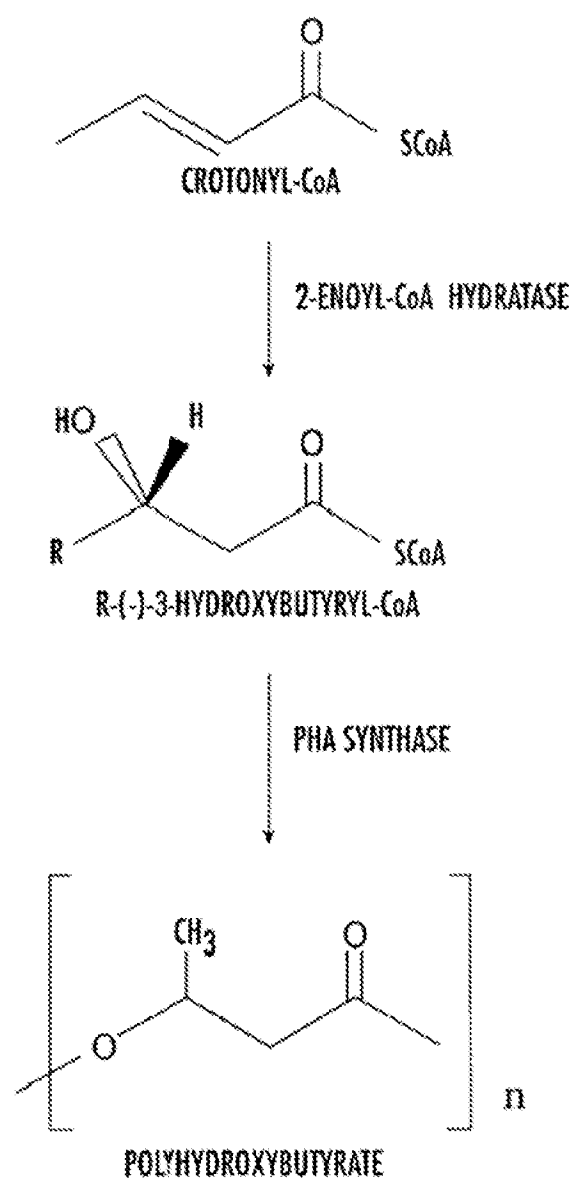


FIG. 4.

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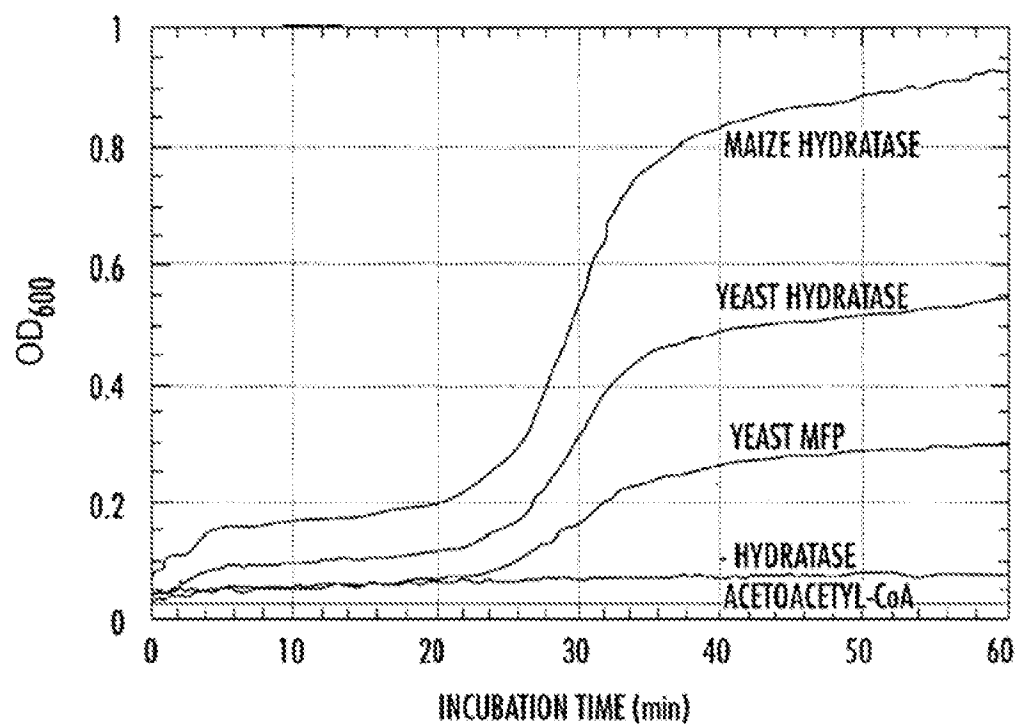


FIG. 5.



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SEQUENCE LISTING

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Li, Chun Ping  
Dong, Jian G  
Hitz, William D  
Liebergesell, Matthias  
Dhugga, Kanwarpal S  
Briggs, Kristen K

<120> PRODUCTION OF POLYHYDROXYALKANOATE IN PLANTS

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<170> PatentIn Ver. 2.1

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agccagcgac tctctctctt gtctctaca agtagccaga caccactccg accttgccgg 180  
caaccgcgtc acagcgacga ggccgagcat aaggcatacg ggcacggcgg cc atg gcg 238  
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5 10 15  
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ctg cat ggt caa caa tac ata gag atc tat agg cca atc cct tgg tat	574
Leu His Gly Gln Gln Tyr Ile Glu Ile Tyr Arg Pro Ile Pro Ser Tyr	
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Val Ser Val Val Asn Arg Val Lys Val Val Gly Leu His Asp Lys Gly	
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35 40 45

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Ile Lys Thr Leu Pro Thr Phe Val Ser Leu Phe Pro Asn Lys Asn Ser  
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195 200 205

Arg Leu Ser Gly Asp Tyr Asn Pro Leu His Ser Asp Pro Asp Ile Ala  
210 215 220

Gln Leu Ala Gly Phe Thr Arg Pro Ile Leu His Gly Leu Cys Thr Leu

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      20           25           30

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370 375 380

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385 390 395 400

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405 410 415

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Cys Thr Leu Gly Ile Ser Ala Lys Ala Leu Phe Glu His Tyr Gly Pro
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465 470 475 480

act cta aag gtt aaa gct tgg aag caa ggc tcg gtt gtc gtt ttt caa 1488
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485 490 495

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Thr Ile Asp Thr Thr Arg Asn Val Ile Val Leu Asp Asn Ala Ala Val
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<223> Nucleotides 4-1566 of SEQ ID NO: 4 corresponds  
to nucleotides 1381-2943 of SEQ ID NO: 3.

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Ser	Phe	Leu	Lys	Met	Lys	Asp	Glu	Glu	Trp	Phe	Ala	Val	Leu	Lys	Val
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His	Leu	Phe	Ser	Thr	Phe	Ser	Leu	Ser	Lys	Ala	Val	Trp	Pro	Ile	Phe
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Thr	Ile	Phe	Ser	Glu	Lys	Glu	Leu	Ser	Asn	His	Phe	Asp	Ala	Ser	Gln
	130					135					140				
Val	Ser	Pro	Leu	Val	Val	Leu	Leu	Ala	Ser	Glu	Glu	Leu	Gln	Lys	Tyr
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Ser	Gly	Arg	Arg	Val	Ile	Gly	Gln	Leu	Phe	Glu	Val	Gly	Gly	Gly	Trp
				165					170						175
Cys	Gly	Gln	Thr	Arg	Trp	Gln	Arg	Ser	Ser	Gly	Tyr	Val	Ser	Ile	Lys
			180					185					190		
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		195					200					205			
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Met	Ala	Thr	Leu	Gln	Ala	Val	Gln	Lys	Ala	His	Ser	Ser	Lys	Glu	Leu
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Asp	Asp	Gly	Leu	Phe	Lys	Tyr	Thr	Thr	Lys	Asp	Cys	Ile	Leu	Tyr	Asn
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Leu	Gly	Leu	Gly	Cys	Thr	Ser	Lys	Glu	Leu	Lys	Tyr	Thr	Tyr	Glu	Asn
			260					265					270		
Asp	Pro	Asp	Phe	Gln	Val	Leu	Pro	Thr	Phe	Ala	Val	Ile	Pro	Phe	Met
		275					280					285			
Gln	Ala	Thr	Ala	Thr	Leu	Ala	Met	Asp	Asn	Leu	Val	Asp	Asn	Phe	Asn
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Tyr	Ala	Met	Leu	Leu	His	Gly	Glu	Gln	Tyr	Phe	Lys	Leu	Cys	Thr	Pro
	305				310					315					320
Thr	Met	Pro	Ser	Asn	Gly	Thr	Leu	Lys	Thr	Leu	Ala	Lys	Pro	Leu	Gln
				325					330					335	



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Val Leu Asp Lys Asn Gly Lys Ala Ala Leu Val Val Gly Gly Phe Glu
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Thr Tyr Asp Ile Lys Thr Lys Lys Leu Ile Ala Tyr Asn Glu Gly Ser
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Gly Lys Arg Ala Lys Phe Ala Val Gln Asn Phe Glu Val Pro His Gly
      385                      390                      395                      400
Lys Val Pro Asp Phe Glu Ala Glu Ile Ser Thr Asn Lys Asp Gln Ala
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Ala Leu Tyr Arg Leu Ser Gly Asp Phe Asn Pro Leu His Ile Asp Pro
      420                      425                      430
Thr Leu Ala Lys Ala Val Lys Phe Pro Thr Pro Ile Leu His Gly Leu
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Cys Thr Leu Gly Ile Ser Ala Lys Ala Leu Phe Glu His Tyr Gly Pro
      450                      455                      460
Tyr Glu Glu Leu Lys Val Arg Phe Thr Asn Val Val Phe Pro Gly Asp
      465                      470                      475                      480
Thr Leu Lys Val Lys Ala Trp Lys Gln Gly Ser Val Val Val Phe Gln
      485                      490                      495
Thr Ile Asp Thr Thr Arg Asn Val Ile Val Leu Asp Asn Ala Ala Val
      500                      505                      510
Lys Leu Ser Gln Ala Lys Ser Lys Leu
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<210> 6  
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 <212> DNA  
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<220>  
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 to nucleotides 241- 2127 of SEQ ID NO: 3.

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gct gga ggg ggc tta ggt aag gta tat gca cta gct tac gca agc aga      96
Ala Gly Gly Gly Leu Gly Lys Val Tyr Ala Leu Ala Tyr Ala Ser Arg
      20                      25                      30

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Gly Ala Lys Val Val Val Asn Asp Leu Gly Gly Thr Leu Gly Gly Ser	
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gga cat aac tcc aaa gct gca gac tta gtg gtg gat gag ata aaa aaa	192
Gly His Asn Ser Lys Ala Ala Asp Leu Val Val Asp Glu Ile Lys Lys	
50 55 60	
gcc gga ggt ata gct gtg gca aat tac gac tct gtt aat gaa aat gga	240
Ala Gly Gly Ile Ala Val Ala Asn Tyr Asp Ser Val Asn Glu Asn Gly	
65 70 75 80	
gag aaa ata att gaa acg gct ata aaa gaa ttc ggc agg gtt gat gta	288
Glu Lys Ile Ile Glu Thr Ala Ile Lys Glu Phe Gly Arg Val Asp Val	
85 90 95	
cta att aac aac gct gga ata tta agg gat gtt tca ttt gca aag atg	336
Leu Ile Asn Asn Ala Gly Ile Leu Arg Asp Val Ser Phe Ala Lys Met	
100 105 110	
aca gaa cgt gag ttt gca tct gtg gta gat gtt cat ttg aca ggt ggc	384
Thr Glu Arg Glu Phe Ala Ser Val Val Asp Val His Leu Thr Gly Gly	
115 120 125	
tat aag cta tgc cgt gct gct tgg cct tat atg cgc tct cag aaa ttt	432
Tyr Lys Leu Ser Arg Ala Ala Trp Pro Tyr Met Arg Ser Gln Lys Phe	
130 135 140	
ggt aga atc att aac acc gct tcc cct gcc ggt cta ttt gga aat ttt	480
Gly Arg Ile Ile Asn Thr Ala Ser Pro Ala Gly Leu Phe Gly Asn Phe	
145 150 155 160	
ggt caa gct aat tat tca gca gct aaa atg ggc tta gtt ggt ttg ggc	528
Gly Gln Ala Asn Tyr Ser Ala Ala Lys Met Gly Leu Val Gly Leu Ala	
165 170 175	
gaa acc ctc gcg aag gag ggt gcc aaa tac aac att aat gtt aat tca	576
Glu Thr Leu Ala Lys Glu Gly Ala Lys Tyr Asn Ile Asn Val Asn Ser	
180 185 190	
att gcg cca ttg gct aga tca cgt atg aca gaa aac gtg tta cca cca	624
Ile Ala Pro Leu Ala Arg Ser Arg Met Thr Glu Asn Val Leu Pro Pro	
195 200 205	
cat atc ttg aaa cag tta gga ccg gaa aaa att gtt ccc tta gta ctc	672
His Ile Leu Lys Gln Leu Gly Pro Glu Lys Ile Val Pro Leu Val Leu	
210 215 220	
tat ttg aca ccc gaa agt acg aaa gtg tca aac tcc att ttt gaa etc	720
Tyr Leu Thr His Glu Ser Thr Lys Val Ser Asn Ser Ile Phe Glu Leu	
225 230 235 240	
gct gct gga ttc ttt gga cag ctc aga tgg gag agg tct tct gga caa	768
Ala Ala Gly Phe Phe Gly Glu Leu Arg Trp Glu Arg Ser Ser Gly Gln	
245 250 255	
att ttc aat cca gac ccc aag aca tat act cct gaa gca att tta aat	816
Ile Phe Asn Pro Asp Pro Lys Thr Tyr Thr Pro Glu Ala Ile Leu Asn	
260 265 270	

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aag tgg aag gaa atc aca gac tat agg gac aag cca ttt aac aaa act	864
Lys Trp Lys Glu Ile Thr Asp Tyr Arg Asp Lys Pro Phe Asn Lys Thr	
275 280 285	
cag cat cca tat caa ctc tcg gat tat aat gat tta atc acc aaa gca	912
Gln His Pro Tyr Gln Leu Ser Asp Tyr Asn Asp Leu Ile Thr Lys Ala	
290 295 300	
aaa aaa tta cct ccc aat gaa caa ggc tca gtg aaa atc aag tcg ctt	960
Lys Lys Leu Pro Pro Asn Glu Gln Gly Ser Val Lys Ile Lys Ser Leu	
305 310 315 320	
tgc aac aaa gtc gta gta gtt acg ggt gca gga ggt ggt ctt ggg aag	1008
Cys Asn Lys Val Val Val Val Thr Gly Ala Gly Gly Gly Leu Gly Lys	
325 330 335	
tct cat gca atc tgg ttt gca cgg tac ggt gcg aag gta gtt gta aat	1056
Ser His Ala Ile Trp Phe Ala Arg Tyr Gly Ala Lys Val Val Val Asn	
340 345 350	
gac atc aag gat cct ttt tca gtt gtt gaa gaa ata aat aaa cta tat	1104
Asp Ile Lys Asp Pro Phe Ser Val Val Glu Glu Ile Asn Lys Leu Tyr	
355 360 365	
ggt gaa ggc aca gcc att cca gat tcc cat gat gtg gtc acc gaa gct	1152
Gly Glu Gly Thr Ala Ile Pro Asp Ser His Asp Val Val Thr Glu Ala	
370 375 380	
cct ctc att atc caa act gca ata agt aag ttt cag aga gta gac atc	1200
Pro Leu Ile Ile Gln Thr Ala Ile Ser Lys Phe Gln Arg Val Asp Ile	
385 390 395 400	
ttg gtc aat aac gct ggt att ttg cgt gac aaa tct ttt tta aaa atg	1248
Leu Val Asn Asn Ala Gly Ile Leu Arg Asp Lys Ser Phe Leu Lys Met	
405 410 415	
aaa gat gag gaa tgg ttt gct gtc ctg aaa gtc cac ctt ttt tcc aca	1296
Lys Asp Glu Glu Trp Phe Ala Val Leu Lys Val His Leu Phe Ser Thr	
420 425 430	
ttt tca ttg tca aaa gca gta tgg cca ata ttt acc aaa caa aag tct	1344
Phe Ser Leu Ser Lys Ala Val Trp Pro Ile Phe Thr Lys Gln Lys Ser	
435 440 445	
gga ttt att atc aat act act tct acc tca gga att tat ggt aat ttt	1392
Gly Phe Ile Ile Asn Thr Thr Ser Thr Ser Gly Ile Tyr Gly Asn Phe	
450 455 460	
gga cag gcc aat tat gcc gct gca aaa gcc gcc att tta gga ttc agt	1440
Gly Gln Ala Asn Tyr Ala Ala Ala Lys Ala Ala Ile Leu Gly Phe Ser	
465 470 475 480	
aaa act att gca ctg gaa ggt gcc aag aga gga att att gtt aat gtt	1488
Lys Thr Ile Ala Leu Glu Gly Ala Lys Arg Gly Ile Ile Val Asn Val	
485 490 495	
atc gct cct cat gca gaa acg gct atg aca aag act ata ttc tcg gag	1536
Ile Ala Pro His Ala Glu Thr Ala Met Thr Lys Thr Ile Phe Ser Glu	
500 505 510	

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Val Leu Leu Ala Ser Glu Glu Leu Gln Lys Tyr Ser Gly Arg Arg Val
      530                      535                      540

att ggc caa tta ttc gaa gtt ggc ggt ggt tgg tgt ggg caa acc aga 1680
Ile Gly Gln Leu Phe Glu Val Gly Gly Gly Trp Cys Gly Gln Thr Arg
545                      550                      555                      560

tgg caa aga agt tcc ggt tat gtt tct att aaa gag act att gaa cgg 1720
Trp Gln Arg Ser Ser Gly Tyr Val Ser Ile Lys Glu Thr Ile Glu Pro
      565                      570                      575

gaa gaa att aaa gaa aat tgg aac cac atc act gat ttc agt cgc aac 1776
Glu Glu Ile Lys Glu Asn Trp Asn His Ile Thr Asp Phe Ser Arg Asn
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act atc aac cgg agc tcc aca gag gag tct tct atg gca acc ttg caa 1824
Thr Ile Asn Pro Ser Ser Thr Glu Glu Ser Ser Met Ala Thr Leu Glu
      595                      600                      605

gcc gtg caa aas gcg cac tct tca aag gag ttg gat gat gga tta ttc 1872
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aag tac act acc aag
Lys Tyr Thr Thr Lys
625

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1887

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<210> 7
<211> 629
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<213> Saccharomyces cerevisiae
<223> Nucleotides 1-1887 of SEQ ID NO: 6 corresponds
      to nucleotides 241- 2127 of SEQ ID NO: 3.

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Gly Ala Lys Val Val Val Asn Asp Leu Gly Gly Thr Leu Gly Gly Ser
      35                      40                      45

Gly His Asn Ser Lys Ala Ala Asp Leu Val Val Asp Glu Ile Lys Lys
      50                      55                      60

Ala Gly Gly Ile Ala Val Ala Asn Tyr Asp Ser Val Asn Glu Asn Gly
      65                      70                      75                      80

Glu Lys Ile Ile Glu Thr Ala Ile Lys Glu Phe Gly Arg Val Asp Val
      85                      90                      95

Leu Ile Asn Asn Ala Gly Ile Leu Arg Asp Val Ser Phe Ala Lys Met

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			115					120					125								
Tyr	Lys	Leu	Ser	Arg	Ala	Ala	Trp	Pro	Tyr	Met	Arg	Ser	Gln	Lys	Phe						
		130				135					140										
Gly	Arg	Ile	Ile	Asn	Thr	Ala	Ser	Pro	Ala	Gly	Leu	Phe	Gly	Asn	Phe						
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Gly	Gln	Ala	Asn	Tyr	Ser	Ala	Ala	Lys	Met	Gly	Leu	Val	Gly	Leu	Ala						
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Glu	Thr	Leu	Ala	Lys	Glu	Gly	Ala	Lys	Tyr	Asn	Ile	Asn	Val	Asn	Ser						
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Ile	Ala	Pro	Leu	Ala	Arg	Ser	Arg	Met	Thr	Glu	Asn	Val	Leu	Pro	Pro						
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His	Ile	Leu	Lys	Gln	Leu	Gly	Pro	Glu	Lys	Ile	Val	Pro	Leu	Val	Leu						
		210				215					220										
Tyr	Leu	Thr	His	Glu	Ser	Thr	Lys	Val	Ser	Asn	Ser	Ile	Phe	Glu	Leu						
		225			230					235					240						
Ala	Ala	Gly	Phe	Phe	Gly	Gln	Leu	Arg	Trp	Glu	Arg	Ser	Ser	Gly	Gln						
				245					250					255							
Ile	Phe	Asn	Pro	Asp	Pro	Lys	Thr	Tyr	Thr	Pro	Glu	Ala	Ile	Leu	Asn						
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Lys	Trp	Lys	Glu	Ile	Thr	Asp	Tyr	Arg	Asp	Lys	Pro	Phe	Asn	Lys	Thr						
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Gln	His	Pro	Tyr	Gln	Leu	Ser	Asp	Tyr	Asn	Asp	Leu	Ile	Thr	Lys	Ala						
		290				295					300										
Lys	Lys	Leu	Pro	Pro	Asn	Glu	Gln	Gly	Ser	Val	Lys	Ile	Lys	Ser	Leu						
		305			310					315					320						
Cys	Asn	Lys	Val	Val	Val	Val	Thr	Gly	Ala	Gly	Gly	Gly	Leu	Gly	Lys						
			325						330					335							
Ser	His	Ala	Ile	Trp	Phe	Ala	Arg	Tyr	Gly	Ala	Lys	Val	Val	Val	Asn						
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Asp	Ile	Lys	Asp	Pro	Phe	Ser	Val	Val	Glu	Glu	Ile	Asn	Lys	Leu	Tyr						
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Gly	Glu	Gly	Thr	Ala	Ile	Pro	Asp	Ser	His	Asp	Val	Val	Thr	Glu	Ala						
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Pro	Leu	Ile	Ile	Gln	Thr	Ala	Ile	Ser	Lys	Phe	Gln	Arg	Val	Asp	Ile						
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Leu	Val	Asn	Asn	Ala	Gly	Ile	Leu	Arg	Asp	Lys	Ser	Phe	Leu	Lys	Met						
				405					410					415							
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Gly Phe Ile Ile Asn Thr Thr Ser Thr Ser Gly Ile Tyr Gly Asn Phe		
450	455	460
Gly Gln Ala Asn Tyr Ala Ala Ala Lys Ala Ala Ile Leu Gly Phe Ser		
465	470	475
Lys Thr Ile Ala Leu Glu Gly Ala Lys Arg Gly Ile Ile Val Asn Val		
485	490	495
Ile Ala Pro His Ala Glu Thr Ala Met Thr Lys Thr Ile Phe Ser Glu		
500	505	510
Lys Glu Leu Ser Asn His Phe Asp Ala Ser Gln Val Ser Pro Leu Val		
515	520	525
Val Leu Leu Ala Ser Glu Glu Leu Gln Lys Tyr Ser Gly Arg Arg Val		
535	535	540
Ile Gly Gln Leu Phe Glu Val Gly Gly Gly Trp Cys Gly Gln Thr Arg		
545	550	555
Trp Gln Arg Ser Ser Gly Tyr Val Ser Ile Lys Glu Thr Ile Glu Pro		
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Glu Glu Ile Lys Glu Asn Trp Asn His Ile Thr Asp Phe Ser Arg Asn		
580	585	590
Thr Ile Asn Pro Ser Ser Thr Glu Glu Ser Ser Met Ala Thr Leu Gln		
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Ala Val Gln Lys Ala His Ser Ser Lys Glu Leu Asp Asp Gly Leu Phe		
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<212> DNA

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<212> DNA

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<400> 17

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